ORIGINAL RESEARCH

Novel Hydrolyzed Chicken Sternal Cartilage Extract Improves Facial Epidermis and Connective Tissue in Healthy Adult Females: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT

Context • Dietary supplement manufacturers claim cutaneous anti-aging properties for their products; however, research supporting these claims remains sparse.
Objectives • The study intended to determine if a correlation existed between the effects of a collagen dietary supplement and changes associated with skin aging.
Design • The study was a 12-week, double-blind, placebo-controlled trial.
Setting • The study took place at a clinical facility specializing in dermatological testing that could perform biophysical, instrumental analysis on the effects of proprietary supplement on human skin.
Participants • Participants were 128 females, aged 39-59 (50.57 ± 5.55).
Intervention • Participants were randomly assigned to an intervention or a placebo. The intervention consisted of twice daily oral administration of a supplement containing 500 mg BioCell Collagen, a chicken sternal cartilage derived dietary ingredient composed of a naturally-occurring matrix of hydrolyzed collagen type-II (≥300 mg), chondroitin sulfate (≥100 mg), hyaluronic acid (≥50 mg).
Outcome Measures • The primary parameters included transepidermal water loss, viscoelasticity, hydration, (indirect) collagen content, chromophore (melanin) content and hemoglobin level, and photographic analysis. An expert visually graded participants' skin to determine the intervention's efficacy, measuring facial lines and wrinkles, crow's feet lines and wrinkles, skin texture and smoothness, and skin tone. The presence of erythema and/or dryness determined tolerance. Secondary outcome measures were tolerance and incidence of adverse events, and the participant's perception of the supplement's value.
Results • For the 113 participants completing the study, the dietary supplementation compared to a placebo: (1) significantly reduced facial lines and wrinkles ($P = .019$) and crow's feet lines and wrinkles ($P = .05$), (2) increased skin elasticity ($P = .008$) and cutaneous collagen content ($P < .001$) by 12%, (3) improved indicators associated with a more youthful skin appearance based on visual grading and wrinkle width ($P = .046$), and (4) decreased skin dryness and erythema. No difference existed between the supplement and the placebo for skin-surface water content or retention. The supplement was well tolerated, with no reported adverse reactions.
Conclusions • Dietary supplementation with chicken, sternal cartilage extract supports the accumulation of types-I/III collagen in skin to promote increased elasticity and reduced skin wrinkling. (*Altern Ther Health Med.* 2019;25(5):12-29.)

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Like all organs in the body, the skin is subject to the aging process, as evidenced by the appearance of an increasing number of wrinkles, sagging and blemishes. Facial skin ages by a combination of intrinsic changes in physiology, including a decline in collagen accumulation and UV-initiated photodamage (photoaging), together with other lifestyle risk factors such as smoking or chronic exposure to environmental pollutants.

Excessive or pronounced UV exposure stimulates photochemical overproduction of radical oxygen species (ROS) and reactive nitrosative species (RNS). These can cause dermal inflammation, immune suppression, and erythema and pigmentation, resulting in dermal and

epidermal degeneration, and can inhibit the expression of procollagen-1. These effects result in elevated levels of degraded collagen and reduction in collagen synthesis that has been implicated in the pathophysiology of human skin aging.[1]

The adrenergic effect of nicotine in cigarettes causes vasoconstriction of blood vessels in the outermost layers of the skin, impairing blood flow, and reducing delivery of nutrients essential to maintaining healthy skin.[2] A single cigarette when smoked has been shown to result in vasoconstriction of the skin for more than 30 minutes,[3] while chronic use can result in tissue hypoxia and damage to elastic fibers and reduced collagen synthesis.[4]

One strategy employed for several decades against these changes has been to administer oral agents to ameliorate the physiological decline of dermal tissue. Among these agents is hydrolyzed collagen used to stimulate dermal fibroblasts; increase collagen synthesis, proteoglycans, and elastin; and improve skin elasticity. A number of clinical studies have examined the effects of hydrolyzed collagen when ingested orally, showing improvements in extracellular matrix synthesis in the dermis as well as in skin microcirculation, enhancement of fibroblast growth, and a reduction in reactive oxidative radicals.[5-13] However, few of these clinical studies have included control groups other than studies by Zague[13] and Proksch et al.[14]

A current approach in the treatment of facial wrinkles is the use of fillers, which are augmentation agents providing aesthetic facial soft tissue. Given the need for repeated treatments with fillers and potential adverse effects on appearance,[15] other approaches have evolved, including treatments that can slow down the processes that contribute to the early appearance of wrinkles and oral agents that might delay the onset or visible appearance of facial wrinkles by noninvasive methods and that can avoid adversely affecting the structure of the skin or injuring the deep dermal plane.[16]

Considerable interest exists in dietary bioactive compounds for their potential to benefit the health and appearance of the skin, especially those compounds that possess a combination of anti-inflammatory, antioxidant, and DNA-repair bioactivities.[17] Natural compounds that benefit facial skin tissue at different skin levels, by mitigating aging processes and supporting the maintenance of dermatological health, would be particularly appealing, especially if conveniently administered orally and demonstrated to be safe.[18,19]

An increasing number of studies have shown that by orally supplying collagen peptides, an increase occurs in the synthesis of connective-tissue components by fibroblasts. The ingestion of gelatin and hydrolyzed collagen derived from animal skin and bones (type-I and type-III collagens), when orally administered to animals and humans, can result in a reduction in the signs of aging, including wrinkles.[20-26] Typically, studies of orally administered mixtures of hydrolyzed collagen type-II (HC-II), hyaluronic acid (HA), and chondroitin sulfate (CS) use different percentages in the compound, rather than a dietary supplement that includes all of them from a single naturally occurring source.

HA is the predominant component of the skin's extracellular matrix, constituting most of the body's total HA, and a key molecule involved in skin moisture owing to its unique capacity to retain water.[27,28] Loss of skin moisture is associated with skin aging. HA has unique rheological, hygroscopic, and viscoelastic properties that make it an appropriate component to support dermal regeneration and augmentation and form hydrated, expanded matrices applicable to cosmeceuticals.[29-31] By combining with glycosaminoglycan to bind and retain water molecules, HA contributes to the skin's pliability, turgor, and resilience.[32] HA has a half-life of 3 to 5 minutes in the blood, less than a day in the skin, and up to 3 weeks in cartilage, with a total normal turnover of 10-100 mg/day in the adult human.[31,33] However, in senescent skin, epidermal HA is virtually undetectable, while in the dermis, it retains its presence.[34]

In a randomized, double-blind, placebo-controlled trial, participants in an intervention group took 2 grams daily of a proprietary supplement for 12 weeks, to relieve symptoms associated with knee and hip osteoarthritis. The supplement contained hydrolyzed-chicken sternal cartilage extract composed of a matrix of collagen type-II peptides (HC-II), HA, and CS. The intervention group experienced statistically significant improvements in their ability to engage in physical activities.[35]

In another randomized, double-blind, placebo-controlled trial, healthy, recreationally active participants took 3 grams of the same proprietary extract over a six-week period, prior to an exercise challenge of upper-body muscle-damaging resistance. The intervention group experienced a favorable improvement in connective and skeletal-muscle tissue biomarkers in addition to enhanced stress resilience after bouts of intense resistance exercise, without any reported side effects.[36]

The decision to perform the current clinical trial of the same proprietary extract to evaluate its ability to reduce the signs of natural and photoaging skin was encouraged by the outcome of an open-label pilot trial of 26 healthy females given one gram of the extract daily for 12 weeks.[10] The pilot study reported that participants experienced, after 12 weeks, a highly significant reduction in skin dryness and scaling ($P = .002$) and significant reduction in global lines and wrinkles ($P = .028$), based on visual and tactile scores.[10] The same study also reported a statistically significant increase in microcirculation of the skin ($P = .018$), based on a 17.7% increase in levels of hemoglobin as well as a highly, statistically significant improvement of 6.3% in the collagen content of the dermis ($P = .002$).

A second open-label pilot study by Hausenblas[37] examined the effects of oral supplementation with 1000 mg of the proprietary extract and 100 mg of trans-resveratrol taken daily for 6 months by 29 women, who displayed visible signs of facial-skin aging. In this study, participants experienced significant reductions in the percentage of facial

pores and ultraviolet spots at the end of 6 months ($P < .05$), based on the use of a device that assessed changes in the skin's surface under normal light, polarized light, and ultraviolet light.[38]

The current study intended to determine if a correlation existed between the effects of a collagen dietary supplement and changes associated with skin aging. As the proprietary extract is a naturally occurring matrix of HC-II, HA, and CS—extracted from chicken sternal cartilage, the current research team conducted a randomized, placebo-controlled trial to evaluate subjective and objective changes in facial cutaneous physiology and corresponding skin appearance in individuals with signs of skin aging. A subgroup of participants, who had been assigned either to the intervention group or a placebo group, were chosen randomly as well to undergo high-resolution image analysis of the facial-line morphologies of crow's feet.

METHODS

This study was a 12-week, randomized, double-blind, placebo-controlled clinical trial.

Participants

The study took place at a clinical facility specializing in dermatological testing that could perform biophysical, instrumental analysis on the effects of proprietary supplement on human skin. The research team recruited 128 healthy females, ages 36 to 59, using the clinical site's patient database. The ethnicity of participants was 99.1% non-Hispanic or Latino and 0.9% Hispanic, with 100% identifying their race as Caucasian.

Participants were included in the study if they: (1) had overall good health; (2) were 36-59 years of age at the time of enrollment; (3) were able and willing to provide consent; (4) fell within the range of Fitzpatrick Skin Types I-IV; (5) showed signs of skin aging, including crow's feet lines ≥2 cm on a 10-cm visual analogue scale (VAS) and facial skin dryness ≥1 on a 5-point ordinal scale, as assessed by expert clinical graders; (6) were free of any dermatologic disorders that might interfere with the evaluation of the proprietary supplement's performance; (7) were willing to abstain from the use of all topical skin-treatment products with claims of skin hydration, lightening, or facial anti-aging, including skin firming, anti-aging, anti-wrinkle, skin lighting, or any topical or systemic medication known to affect skin aging or dyschromia—products containing alpha/beta/poly-hydroxy acids, vitamin C, soy, CoQ-10, hydroquinone—or systemic or topical retinoids; and (8) were willing to arrive at the test center for the study's scheduled visits, prior to which they would apply no topical treatments, including moisturizers and creams.

Recruited individuals were excluded from participation if they: (1) were participating in another clinical study; (2) had an underlying disease or surgical or medical condition that could put them at risk in the opinion of the principal investigator, including any uncontrolled chronic or serious disease that would normally prevent participation in any clinical trial, such as a cancer, AIDS, diabetes, obesity, renal impairment, or mental illness; (3) had an addiction to drugs and/or alcohol; (4) had a history of self-reported allergies to cosmetic and/or sunscreen products; (5) were immunocompromised; (6) had previously used any dietary supplement containing collagen, HA, CS, or any combination thereof within the 3 months prior to recruitment; (7) had undergone recent, anti-aging facial procedures within the 3 months prior to recruitment, including superficial to mid-deep chemical peels, (8) had undergone dermabrasion within the 6 months prior to the screening visit; (9) had undergone facial plastic surgery or ablative-laser or fractional-laser resurfacing of facial skin within the 12 months prior to recruitment; (10) had undergone a regimen of Thermage treatments or an equivalent type of high-energy treatment on the face within the 12 months prior to recruitment; (11) had a history of allergic reactions to any ingredient in the tested supplement; (12) had started hormone replacement therapy within the 3 months preceding the screening visit; (13) had used oral contraceptives for fewer than 3 months prior to the screening visit; had changed contraceptive method within the 3 months prior to the screening visit; or planned to modify contraception treatment within the duration of the study; (14) were pregnant, nursing, or had plans to become pregnant within 6 months; or (15) were unable to communicate or cooperate due to language problems, poor mental development, or impaired cerebral function. Employees of the clinical lab or other testing firms or laboratories as well as those of cosmetic or raw-material manufacturers of topical products or their suppliers were excluded from participation.

The study was approved by an Institutional Review Board (Allendale IRB, Old Lyme, CT; #3757BT1013) and conducted in accordance with US FDA Good Clinical Practices guidelines (21 CFR 50.25). The research team obtained written informed consent of all participants in accordance with guidelines of the International Conference on Harmonization[39] for the evaluation of the efficacy of cosmetic products.[40] Participants were advised to report all adverse experiences to the study's personnel in a timely manner, whether or not they considered them to be supplement related. Because the study was not intended for submission to the FDA as part of an IND application, and the intervention is a dietary supplement as defined under 21 CFR 58, it was not registered with Clinicaltrials.gov.

Procedures

Before the beginning of the study, participants were instructed to use the following products to clean and/or moisturize the skin throughout the duration of the study: Camay bar soap and Cetaphil Facial Wash or Cetaphil Moisturizer. Participants were allowed continued use of coloring facial cosmetics that made no anti-aging claims that they had used regularly prior to enrollment into the study.

During the baseline visit, potential participants arrived following their morning facial washing after which they acclimated to the testing environment for at least 15 minutes.

Table 1. Summary of the Study's Procedures

Procedure	Consent/Screening	Baseline	Week 6	Week 12
Consent and medical history	X			
Inclusion/exclusion criteria reviewed	X	X		
Dispense (D)/collect (C) products and review diaries	D[a]	D	D	C
Diary review, product accountability			X	C
Ordinal assessments for dryness and erythema		X		X
Expert grader assessment for efficacy: global lines/wrinkles; crows' feet lines/wrinkles; texture/softness; tone		X	X	X
Subjective questionnaire			X	X
Skin-surface hydration—MoistureMeterSC		X	X	X
Skin-collagen content—SIAScope		X	X	X
Transepidermal water loss (TEWL)— Vapometer		X	X	X
Elasticity—Cutometer MPA 580		X	X	X
Photographic analysis—Clarity 2D Research Ti		X	X	X

[a]Washout soap dispensed

Following participant's passing of the inclusion and exclusion criteria, an expert then graded their skin using ordinal visual grading for skin dryness, erythema, global lines and wrinkles, crow's feet, texture, and tone. Instrumental analyses were performed to establish baseline values.

The clinical trial included 4 visits carried out over a period of 12 weeks. It was performed by a contract research organization (CRO), International Research Services (IRSI), in Port Chester, NY. The CRO specializes in dermatological safety and efficacy testing and is located at latitude 41.0018 degrees (Westchester County, New York, USA). The IRSI trial was initiated in mid-May and concluded in mid-November and included one visit for consenting, screening and qualification procedures and washout instructions (one-week washout period, 12-week treatment period), a second visit for baseline pre-dosing evaluations, a third assessment visit at Week 6, and a final assessment visit at the end of the study in Week 12. An outline of the study's procedures is shown in Table 1.

All instrumental tests were controlled by qualified professionals trained in the skilled use of each apparatus. All investigators and participants were blinded as to who received the intervention and the placebo. To prevent selection bias, randomization was performed by random block size to group allocation using randomization.com.

All supplements in the study were labeled with appropriate codes and use instructions. Each capsule of the intervention supplement used BioCell Collagen from BioCell Technology (Anaheim, CA). These capsules contained 300 mg of hydrolyzed collagen type-II (HC-II); 100 mg of glycosaminoglycan (GAG), chondroitin sulfate (CS); and 50 mg of hyaluronic acid (HA). Each of its soluble components were derived from chicken sternal cartilage. The placebo contained 500 mg of cellulose per capsule.

The supplement and its components were tested for purity using the following methods: the AOAC Method 992.15 for host cell protein (HCP).[41] United States Pharmacopeia (USP) method for CS (chondroitin sulfate sodium, [United States Pharmacopeia, Revised Bulletin USP 39-NF 34, June 1, 2015]); and high-performance gel permeation chromatography (GPC) for HA according to the method of Motohashi et al.[42] Inactive ingredients in the supplement include gelatin, rice flour, and magnesium stearate. Studies have shown that the active components of the supplement, when administered orally, are distributed into tissues following absorption, including the skin.[43-45] The research team subjectively and objectively assessed the efficacy of daily oral administration of the proprietary extract in improving markers of skin aging. The secondary objective was to assess the tolerance for the intervention when taken according to the manufacturer's instructions.

Intervention

Participants were instructed to ingest one capsule in the morning and another in the evening with a full glass of water, preferably on an empty stomach. Fifty-eight participants were assigned to the intervention group, while 56 participants were assigned to the placebo group, by randomized assignment. The demographics of participants who completed the study in each group are shown in Table 2.

Outcome Measures

Dryness and Erythema. Dryness was assessed on an ordinal scale from 0 to 4, with grade 0 = smooth—no signs of dryness, 1 = mild—slight but definite dryness, fine scales present, possible powdery or ash-appearance, 2 = moderate—mild flaking, some uplifting scales, 3 = moderate to severe—peeling, flaking scales, and 4 = severe—evident and profuse flaking and very coarse scaling, cracking progressing to fissuring, and possible erythema and/or edema. Erythema was assessed on a scale from 0 to 4, with 0 = no erythema, 1 = very slight erythema that is barely perceptible, 2 = well-defined erythema, 3 = moderate to severe erythema, and 4 = severe erythema (beet redness) to slight eschar formation (injuries in depth).

Table 2. Participants' Demographics

Variable	Intervention Group			Placebo Group		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Age, y	51.15 ± 5.32	41	59	50.87 ± 5.62	36	59
Height, inches	63.55 ± 2.06	60	69	64.67 ± 2.51	58	70
Weight, pounds	146.55 ± 28.51	108	230	153.98 ± 39.05	100	300
Ethnicity	Hispanic	0	0.0		1	1.8
	Not Hispanic or Latino	58	100		54	98.2
Race	White	58	100		55	100
Fitzpatrick skin type	Skin Type I	4	6.9		3	5.5
	Skin Type II	15	25.9		14	25.5
	Skin Type III	26	44.8		29	52.7
	Skin Type IV	13	22.4		9	16.4
Facial skin type	Combination	33	56.9		21	38.2
	Dry	6	10.3		10	18.2
	Normal	19	32.8		23	41.8
	Oily	0	0.0		1	1.8

Note: the intervention group (n = 58) and the placebo group (n = 55)

Abbreviations: SD, standard deviation.

Expert Clinical Evaluations. This assessment, by expert grading—that included evaluating facial lines and wrinkles, crow's feet lines and wrinkles, texture and smoothness, and skin tone—used a validated visual analog scale (VAS) of 10-cm bars with horizontal lines defining the extreme limits, oriented from left (best) to right (worst).[46] Graders were trained for repetition and ability to detect changes, and whenever possible, the same graders evaluated the same participants during subsequent visits. Lighting was kept consistent by eliminating outside lighting and providing light-magnifying lenses. A single site and 3 measurements were averaged, and a global face map was used to ensure proper evaluation for each region at each time point.

Transepidermal Water Loss. Transepidermal water loss (TEWL) was assessed in duplicate using the portable VapoMeter (Delfin Technologies, Kuopio, Finland), a validated instrument for the measurement of transepidermal water loss values and evaporation rates.[47] The device uses a closed cylindrical chamber that contains sensors for relative humidity and temperature. Surface water is a function of water lost through the epidermis that provides an indication of the integrity of the skin barrier present in the stratum corneum. Changes in TEWL rates provide a measure of barrier disruption or integrity, thereby providing an indication of supplement performance.

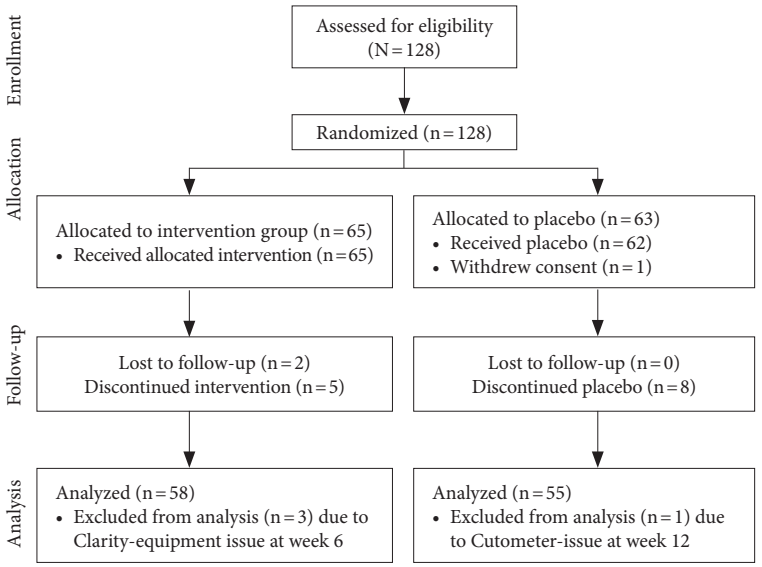
When the Vapometer is in contact with the skin, the relative humidity % (RH %) in the chamber begins to increase. The TEWL value is calculated from the information the instrument acquires based on this increase. The measurement range is from 3 to 200 g/m^{2}h; the closed chamber allows for accuracy of measurement range in these high values. Duplicate measurements were collected on the right or left cheek according to a randomized schedule, performed at baseline and weeks 6 and 12, with the average recorded of each measurement.

Skin-Surface Hydration. To quantify the level of skin-surface hydration, a handheld MoistureMeterSC (Delfin Technologies, Kuopio, Finland), was used. It's a validated instrument[48] employed to measure the capacitance of the layered structure of the skin composed of the, stratum corneum and underlying layers. The measured capacitance is directly proportional to the water content of the stratum corneum and is found to be sensitive in measuring the hydration of the stratum corneum.[48] The portable unit is capable of taking 12 measurements per minute using a built-in pressure sensor. The results are graphically displayed, and data were transmitted wirelessly and accounted for the changing thickness of the stratum corneum's dry layer. Measurements were taken in duplicate of a participant's left or right check according to a randomization code performed, at baseline and again at weeks 6 and 12, with the average recorded of each measurement.

Elasticity. Elastin (elasticin) is a key extracellular matrix protein found in skin, connective tissues, and internal organs, together with collagen. It contributes to the viscoelasticity of the skin.[49] Elasticity defines the ability of the skin to change its shape under application of a force and to restore it to its original shape when the force is removed. Natural and photoaged skin is characterized by degradation and alteration of the sum of disorganized elastin and the alteration of collagen fibers, which provide structure, throughout the dermis, a condition known as elastosis. Hence, the skin's viscoelastic properties can be a quantifiable indicator of the biological age of the skin. Viscoelasticity of each participant's skin was determined using a validated device, the Cutometer MPA 580 (Courage + Khazaka, Cologne, Germany). It measures the viscoelastic properties of the skin by applying suction to the skin surface, drawing the skin into the aperture of the probe and determining the penetration depth using an optical measuring system.[50,51] Cutometer measurement of all participants were taken in the same location on their left and right check area, according to use of a randomization code assigned at baseline and at the visits in the sixth and twelfth weeks. Skin firmness was reported using the RO (Uf) parameter. As the skin becomes firmer, this value decreases, while skin elasticity was reported using the R5 (Ur/Ue net elasticity) parameter. The skin becomes more elastic as this value increases.

Collagen Content. To evaluate skin-collagen content, skin imaging of collagen content and mapping were carried out using a Cosmetrics SIAscope (Astron Clinica, Toft, UK).[52] The SIAscope uses spectrophotometric intracutaneous analysis (SIA), known as chromophore mapping, that is achieved by employing a combination of dermatoscopy and contact remittance spectrophotometry. Cosmetrics can quantify changes in blood, melanin, and collagen concentration up to 2 mm below the skin's surface. In

Figure 1. Consort Flow Diagram



primary-care assessment studies using the SIAscope to determine the sensitivity and specificity of the device, its sensitivity was reported to be 94.4%, with a false negative rate of 3.7%[53]; its ability to accurately identify melanin, hemoglobin, and collagen was found to be extremely high[54]; and sensitivity and specificity to detect melanoma, has been reported to be 80.1%, and 82.7%, respectively.[55]

The device performs statistical analysis on each chromophore, tracking changes over time and allowing the operator to visualize and validate the performance of a product with high reproducibility. Using the handheld imaging probe, attached to a laptop computer, the unit is placed in contact with the skin surface and high-intensity LED's illuminate the skin at discreet wavelengths of 200 to 1000 nm, spanning the visible spectrum and a small range of the near infrared spectrum. A digital image is captured for each wavelength. Three parametric chromophore maps were retrieved up to 2 mm in depth and 11 mm in circumference, one for each of the following parameters: epidermal melanin, dermal hemoglobin, and dermal collagen. Measurements were taken at baseline, during the visit at 6 weeks, and upon conclusion of the study at Week 12, on the left and right cheek of each participant. according to a randomized schedule

Photographic Analysis. Photographic analysis was carried out with the Clarity 2D Research Systems Ti (Brightex Bio-Photons, San Jose, CA) using a high-quality, 18-megapixel, digital imaging device and colorimeter (Minolta Chroma Meter CR-400, Ramsey, NJ). The Clarity device captures high-quality face frontal and left and right lateral images. Multi-spectral diffuse white light, cross-polarized blue, and parallel polarized lighting reveals skin conditions on and beneath the skin's surface layer. Images were analyzed for attributes associated with pigmentation, radiance, skin color, redness, wrinkles, skin texture, pores, acne and lips. Images of 20 participants in the intervention group and 17 in the placebo group were captured of full frontal, and left and right lateral directions, in addition to capturing images for crow's feet and wrinkles' width. To prepare participants for photography, the research team asked them to remove all jewelry that might be visible when images were taken, and they were provided a black headband and cape. The photography took place in a designated suite with a black matte background conducive to clinical booth photography in which natural light is blocked out.

Participants' Perceptions. To gauge the perceived value of using the supplement, a questionnaire was administered to participants during their visit at Week 6 and again at the final visit in Week 12. The questionnaire consisted of 7 statements that participants rated on a scale of 1 to 5, related to the use of the supplement they used. Subjective ratings included statements about the appearance of lines and wrinkles on the entire face, crow's feet, skin tone, skin texture and smoothness, facial skin hydration, and irritation and redness caused by dryness.

Statistical Analysis

Analysis of the data included that of all participants randomly assigned to either group and adjusted for normality distribution. Descriptive statistics included analysis of data with clinical grading for efficacy, image analysis, and instrumental assessments, using a paired *t* test to compare results from both visits to baseline and an unpaired t-test to compare the intervention to placebo. All continuous variables were captured as means ± standard deviations (SDs). Treatment effects were defined as weighed mean differences and 95% confidence intervals between the intervention and control groups. The supplement questionnaire was analyzed by the Mann-Whitney *U* Test. Statistical significance was set at $P \leq .05$, and all analyses were performed using SPSS (Version 11.6, IBM, Armonk, NY).

RESULTS

A flow diagram of the trial is shown in Figure 1. Of the 128 individuals recruited for the study, 65 were randomly assigned to the intervention group and 63 to the placebo group. Of the 128 individuals who participated in the study, five withdrew consent, two failed to appear for assessment at week-6, five were dropped due to suspected noncompliance, and three were dropped out of the study due to scheduling conflicts, a dropout rate of 12%.

Compliance was also measured by pill counts at each visit and review of participants' diaries. Noncompliance was predetermined if a participant consumed less than 80% of the required pills based on a count of the remaining pills at each visit. Of the 15 participants that dropped out of the study, no adverse effects were reported, or by those participants that completed the study.

Table 3. Analysis of Skin Dryness, Erythema, and Transepidermal Water Loss

		Intervention Group			Placebo Group		
Assessment	**Time Point**	**n**	**Mean ± SD**	***P* Value**	**n**	**Mean ± SD**	***P* Value**
Dryness	Baseline	58	1.94 ± 0.43		55	1.96 ± 0.47	
	Week 12	58	0.06 ± 0.25	<.001[a]	55	0.14 ± 0.40	<.001[a]
Erythema	Baseline	58	0.37 ± 0.58		55	0.41 ± 0.62	
	Week 12	58	0.17 ± 0.42	.002*	55	0.32 ± 0.51	.005
TEWL	Baseline	58	14.69 ± 4.18		55	15.91 ± 4.00	
	Week 6	57	14.14 ± 2.86	.240	55	16.03 ± 4.97	.850
	Week 12	58	15.00 ± 4.05	.609	55	16.33 ± 5.44	.576

[a]Indicates a statistically significant improvement compared to baseline, $P \leq .05$

Abbreviations: SD, standard deviation; TEWL, transepidermal water loss.

Table 4. Subjective Changes in Dryness and Erythema from Baseline to Week 12

			Intervention Group					Placebo Group			
Assessment	**Time Point**	**n**	**Mean ± SD**	**Mean % Improvement From Baseline Mean**	**% of Participants with Improvement**	***P* Value**	**n**	**Mean ± SD**	**Mean % Improvement From Baseline Mean**	**% of Participants with Improvement**	***P* Value**
Dryness	Baseline	58	1.94 ± 0.43				55	1.96 ± 0.47			
	Week 12	58	0.06 ± 0.25	96.55	100	<.001[a]	55	0.14 ± 0.40	91.81	96.4	<.001[a]
Erythema	Baseline	58	0.37 ± 0.58				55	0.41 ± 0.62			
	Week 12	58	0.17 ± 0.42	60.52	20.7	.002[a]	55	0.32 ± 0.51	47.36	20.0	.255

[a]Indicates a statistically significant improvement compared to baseline, p≤0.05

Abbreviations: SD, standard deviation

Table 5. Between Group Analysis for Dryness and Erythema

			Intervention Group		Placebo Group	
Assessment	**Time Point**	**n**	**Mean Difference ± SD From Baseline**	**n**	**Mean Difference ± SD From Baseline**	***P*$_T$ Value**
Dryness	Week 12	58	-1.87 ± 0.49	55	-1.81 ± 0.64	0.574
Erythema	Week 12	58	-0.20 ± 0.48	55	-0.09 ± 0.58	0.256

Abbreviations: SD, standard deviation

Table 6. Subjective Change in Scores for Dryness and Erythema from Baseline to Week 12

			Intervention Group						Placebo Group				
			Frequency of Score N (%)						Frequency of Score N (%)				
Assessment	**Time Point**	**n**	**0**	**1**	**2**	**3**	**4**	**n**	**0**	**1**	**2**	**3**	**4**
Dryness	Baseline	58	0 (0.0)	7 (12.1)	47 (81.0)	4 (6.9)	0 (0.0)	55	0 (0.0)	7 (12.7)	43 (78.2)	5 (9.1)	0 (0.0)
	Week 12	58	54 (93.1)	4 (6.9)	0 (0.0)	0 (0.0)	0 (0.0)	55	48 (87.3)	6 (10.9)	1 (1.8)	0 (0.0)	0 (0.0)
Erythema	Baseline	58	39 (67.2)	16 (27.6)	3 (5.2)	0 (0.0)	0 (0.0)	55	36 (65.5)	15 (27.3)	4 (7.3)	0 (0.0)	0 (0.0)
	Week 12	58	49 (84.5)	8 (13.8)	1 (1.7)	0 (0.0)	0 (0.0)	55	38 (69.1)	16 (29.1)	1 (1.8)	0 (0.0)	0 (0.0)

Abbreviations: SD, standard deviation

Table 7. Differences in Changes in Facial Lines, Wrinkles, Crow's Feet, Texture, Smoothness, and Skin Tone, by Expert Evaluators.

			Intervention Group					Placebo Group			
Assessment	Time Point	n	Mean ± SD	Mean % Improvement From Baseline Mean	% of Participants with Improvement	*P* Value	n	Mean ± SD	Mean % Improvement From Baseline Mean	% of Participants with Improvement	*P* Value
Facial Lines/ Wrinkles	Baseline	58	5.67 ± 0.88				55	5.40 ± 1.05			
	Week 6	57	5.57 ± 0.87	1.07	50.9	.162	55	5.47 ± 1.06	NI	41.8	.322
	Week 12	58	5.19 ± 0.93	8.35	79.3	<.001[a]	55	5.24 ± 0.85	0.63	61.8	.177
Crow's Feet Lines/ Wrinkles	Baseline	58	5.65 ± 1.19				55	5.25 ± 1.22			
	Week 6	57	5.42 ± 1.15	3.70	68.4	.002[a]	55	5.01 ± 1.23	4.12	60.0	.004[a]
	Week 12	58	4.99 ± 1.16	10.59	79.3	<.001[a]	55	4.94 ± 1.18	4.07	54.5	.027[a]
Texture/ Smoothness	Baseline	58	6.15 ± 1.13				55	6.40 ± 1.16			
	Week 6	57	4.48 ± 1.38	20.67	80.7	<.001[a]	55	5.21 ± 1.23	17.19	80.0	<.001[a]
	Week 12	58	4.19 ± 1.07	29.52	91.4	<.001[a]	55	4.36 ± 0.99	28.40	87.3	<.001[a]
Skin Tone	Baseline	58	6.02 ± 1.02				55	5.94 ± 0.92			
	Week 6	57	5.71 ± 0.98	4.15	64.9	<.001[a]	55	5.47 ± 0.98	7.69	70.9	<.001[a]
	Week 12	58	5.29 ± 1.04	10.74	70.7	<.001[a]	55	5.32 ± 0.97	9.81	70.9	<.001[a]

[a]$P \leq .05$

Abbreviations: SD, standard deviation; NI, no improvement.

Table 8. Group Comparisons for Subjective Changes in Facial Lines, Wrinkles, Crow's Feet, Texture, Smoothness, and Skin Tone

			Intervention Group		Placebo Group	
Assessment	Time Point	n	Mean Difference ± SD From Baseline	n	Mean Difference ± SD From Baseline	P_T Value
Facial Lines/Wrinkles	Week 6	57	-0.07 ± 0.40	55	0.07 ± 0.53	.103
	Week 12	58	-0.48 ± 0.53	55	-0.15 ± 0.85	.019[a]
Crow's Feet Lines/ Wrinkles	Week 6	57	-0.23 ± 0.55	55	-0.23 ± 0.56	.956
	Week 12	58	-0.65 ± 0.89	55	-0.30 ± 0.99	.051
Texture/ Smoothness	Week 6	57	-1.30 ± 1.23	55	-1.19 ± 1.24	.632
	Week 12	58	-1.95 ± 1.43	55	-2.04 ± 1.64	.757
Skin Tone	Week 6	57	-0.28 ± 0.55	55	-0.47 ± 0.65	.103
	Week 12	58	-0.72 ± 0.97	55	-0.62 ± 0.87	.566

[a]The intervention outperformed the placebo, $P \leq .05$.

Abbreviations: SD, standard deviation.

Dryness and erythema. Statistically significant changes were seen for skin dryness at Week 12 for both the intervention and placebo groups, when compared to baseline, and for erythema for the intervention group only (Table 3). No statistical change was seen for transepidermal water loss at Week 6 or at Week 12 for either group, compared to baseline.

A statistically significant mean percentage improvement occurred in skin dryness from baseline to Week 12 ($P < .001$) in both groups, but it disappeared when the data were analyzed for between-group differences (Table 4). While the intervention group experienced a significant improvement in erythema after 12 weeks ($P < .002$) no significant differences existed between groups for either dryness or erythema (Table 5). The percentage frequency of scores for dryness and erythema for each group is shown in Table 6.

Expert Clinical Evaluations. These evaluations for facial lines and wrinkles, crow's feet, texture and smoothness, and skin tone, showed statistically significant improvements between baseline and Week 12 for the intervention group and for crow's feet, texture and smoothness, and skin tone for the placebo group (Table 7). A significant difference was seen for the number of facial lines and wrinkles between the intervention group and the placebo group at Week 12 (Table 8). No significant differences existed between the groups at Week 12 for crow's feet, texture and smoothness, and skin tone.

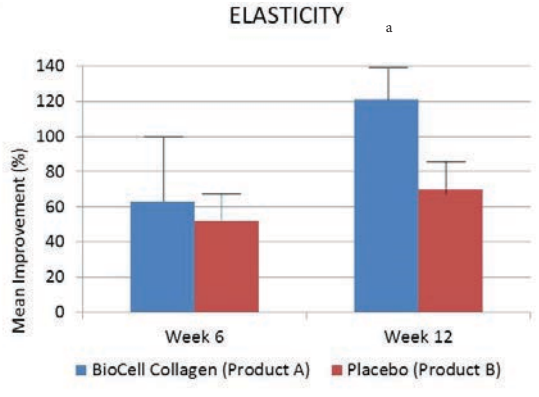
Table 9. Objective Instrument Driven Changes in Facial Lines, Wrinkles, Crow's Feet, Texture, Smoothness, and Skin Tone for Both Groups

Assessment		Time Point	Intervention Group n	Intervention Group Mean ± SD	Intervention Group Mean % Improvement From Baseline Mean	Intervention Group % of Participants with Improvement	Intervention Group *P* Value	Placebo Group n	Placebo Group Mean ± SD	Placebo Group Mean % Improvement From Baseline Mean	Placebo Group % of Participants with Improvement	Placebo Group *P* Value
VapoMeter (TEWL)		Baseline	58	14.69 ± 4.18				55	15.91 ± 4.00			
		Week 6	57	14.14 ± 2.86	NI	52.6	.240	55	16.03 ± 4.97	NI	50.9	.850
		Week 12	58	15.00 ± 4.05	NI	50.0	.609	55	16.33 ± 5.44	NI	50.9	.576
Moisture Meter		Baseline	58	41.84 ± 29.19				55	39.59 ± 23.95			
		Week 6	57	62.36 ± 25.49	148.95	80.7	<.001[a]	55	64.93 ± 24.11	163.69	74.5	<.001[a]
		Week 12	58	61.31 ± 24.53	145.86	70.7	<.001[a]	55	62.08 ± 28.41	159.32	69.1	<.001[a]
Cutometer	Firmness R0 (Uf)	Baseline	58	0.08 ± 0.09				55	0.16 ± 0.07			
		Week 6	57	0.08 ± 0.09	NI	43.9	.861	55	0.19 ± 0.09	NI	32.7	.003[b]
		Week 12	58	0.07 ± 0.07	NI	53.4	.066	54[c]	0.19 ± 0.10	NI	42.6	.009**
	Elasticity R5 (Ur/Ue)	Baseline	58	0.20 ± 0.07				55	0.22 ± 0.06			
		Week 6	57	0.30 ± 0.08	67.71	78.9	<.001[a]	55	0.31 ± 0.10	54.66	69.1	<.001[a]
		Week 12	58	0.40 ± 0.08	124.50	94.8	<.001[a]	54[c]	0.34 ± 0.15	72.01	72.2	<.001[a]
SIAScope	Collagen	Baseline	58	176.27 ± 17.89				55	178.54 ± 20.15			
		Week 6	57	169.88 ± 13.15	NI	38.6	.002[b]	55	166.47 ± 14.04	NI	27.3	<.001**
		Week 12	58	196.59 ± 25.49	11.73	86.2	<.001[a]	55	147.34 ± 37.95	NI	7.3	<.001**
	Hemoglobin	Baseline	58	98.76 ± 16.88				55	102.21 ± 21.27			
		Week 6	57	99.96 ± 20.19	NI	45.6	.565	55	98.12 ± 17.84	2.02	58.2	.088
		Week 12	58	91.66 ± 45.01	4.40	93.1	.262	55	98.36 ± 49.67	NI	74.5	.606
	Melanin	Baseline	58	158.02 ± 22.97				55	159.74 ± 24.87			
		Week 6	57	151.32 ± 21.90	4.05	71.9	.002[a]	55	148.14 ± 21.04	6.49	76.4	<.001[a]
		Week 12	58	147.90 ± 33.18	4.92	43.1	.048[a]	55	139.90 ± 29.09	10.45	56.4	<.001[a]

[a]Indicates a statistically significant improvement compared to baseline, $P \leq .05$
[b]Indicates a statistically significant worsening compared to baseline, $P \leq .05$
[c]One subject's (#17) data was not captured at the Week 12 visit due to a technical issue for R0 and R5 (54 participants' data analyzed)

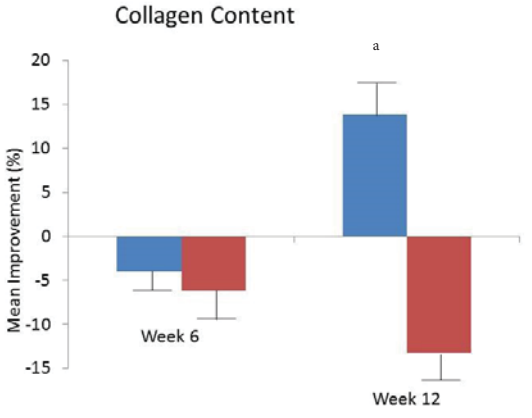
Abbreviations: SD, standard deviation; TEWL, transepidermal water loss; NI, no improvement.

Figure 2. Skin elasticity—Mean percentage change from baseline. The data were obtained using the Cutometer bioinstrumentation data (R0). The figure shows 12-week group comparisons.



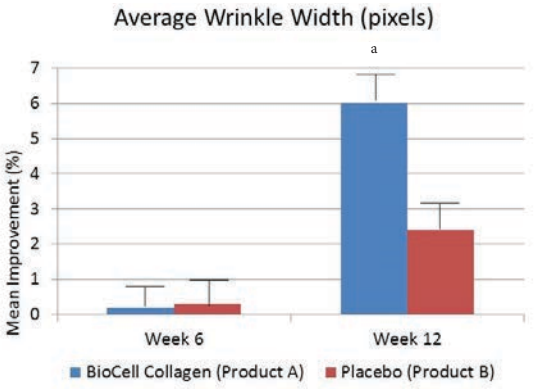
[a]$P < .05$ compared to placebo

Figure 3. Average Collagen content. The blue bars indicate the intervention group; the red bars indicate the placebo group. The figure shows the mean percentage change from baseline. The data were obtained using the Siascope bioinstrumentation. The figure shows 12-week group comparisons.



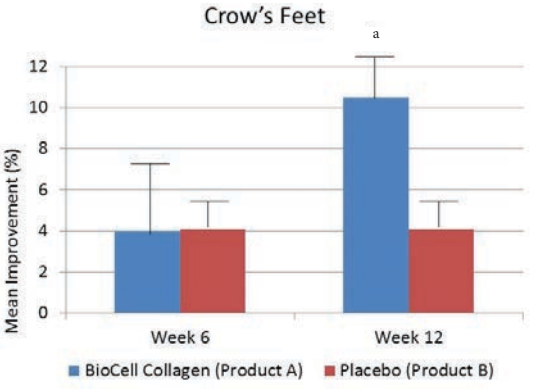
[a]$P < .05$ compared to placebo

Figure 4. Average wrinkle width: Mean percentage change from baseline for a 46-year old female. The data were obtained using Clarity photography image analysis. The figure shows 12-week group comparisons.



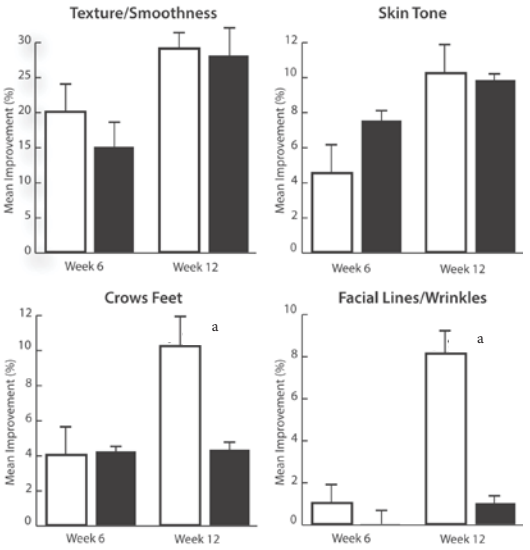
[a]$P < .05$ compared to placebo

Figure 5. Crow's feet wrinkles: The blue bars indicate the intervention group; the red bars indicate the placebo group. Mean percentage change from baseline for a 58-year old female. The data were obtained using expert visual grading. The figure shows 12-week group comparisons.



[a]$P < .05$ compared to placebo

Figure 6. Changes in texture/smoothness, skin tone, crow's feet, and facial lines/wrinkles from Week 6 to Week 12.



[a]$P < .05$ compared to placebo

Photographic Analysis. Photographic evidence of facial improvements in the intervention group are illustrated in the photos shown for three participants: the reduction in eyelines and wrinkles, and facial sagging, as seen in a 59-year old female (Figure 7); reduction in crow's feet lines and wrinkles for this 55-year old female (Figure 8); and, the reduction of both lines and wrinkles, and improved radiance, in a 47-year old female (Figure 9).

TEWL. No significant improvements occurred in either the intervention or the placebo group for hemoglobin and transepidermal water loss based on measurements taken using the VapoMeter and the SIAScope, respectively (Table 9). Statistically significant improvements occurred in skin moisture for both the intervention and placebo groups at Week 12 compared to baseline.

Elasticity and Collagen Content. No statistically significant changes were seen between baseline and Week 12 in skin firmness in the intervention group. However, a statistically significant improvement had occurred for the intervention group in skin elasticity at 12 weeks, 0.40 ± 0.08, compared to baseline (0.20 ± 0.0), $P < .001$ (Figure 2). Furthermore, collagen also experienced a statistically significant improvement for the intervention group ($P < .001$) by the end of the study (Figure 3) In contrast, the placebo group experienced a significant worsening in collagen, $P < .001$, and significant improvement in elasticity ($P = .006$).

Average Wrinkle Width. For the intervention group, a statistically significant improvement in average wrinkle width occurred (Figure 4) as well as in the appearance of facial lines and wrinkles and crow's feet when comparing groups (Figure 5).

Figure 7. Baseline and week-12 (right) profiles of a 59-year old female displaying a reduction in lines/wrinkles and radiance.

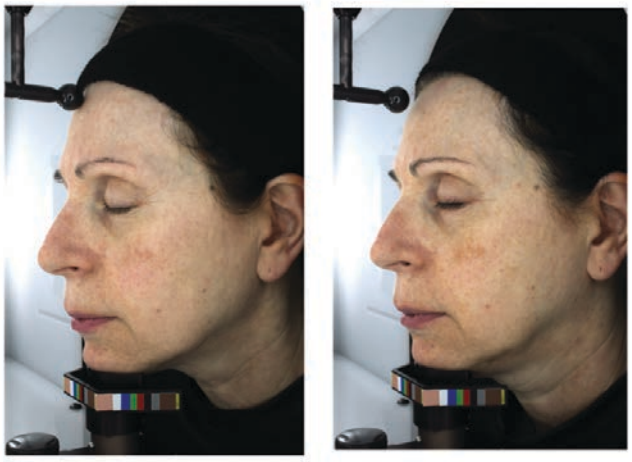


Figure 8. Baseline and week-12 (right) profiles of a 55-year old female illustrating the reduction in both crows feet lines and wrinkles.

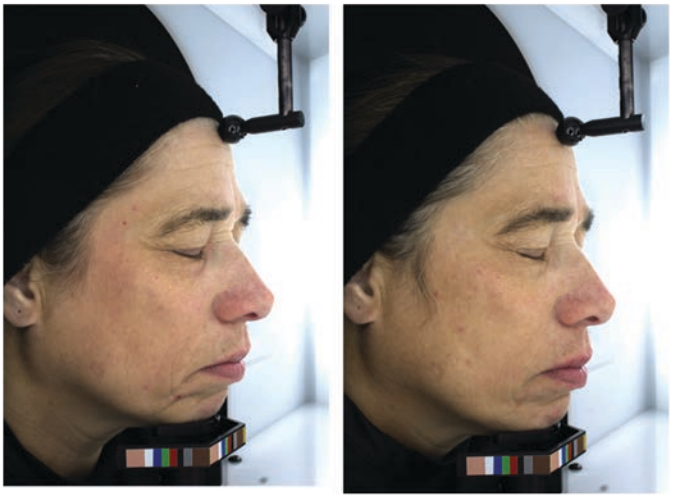


Figure 9. Baseline and week-12 (right) profiles of a 47-year old female showing the reduction in lines and wrinkles, and improved radiance.

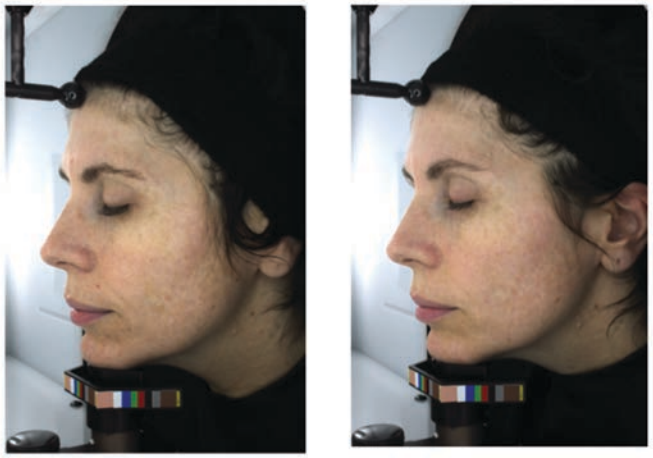


Table 10. Intergroup Objective Changes in Skin Based on Instrumentation Assessments

Assessment		Time Point	Intervention Group n	Intervention Group Mean Difference ± SD From Baseline	Placebo Group n	Placebo Group Mean Difference ± SD From Baseline	P_T Value
VapoMeter (TEWL)		Week 6	57	-0.58 ± 3.69	55	0.11 ± 4.61	.379
		Week 12	58	0.31 ± 4.70	55	0.42 ± 5.55	.914
Moisture Meter		Week 6	57	20.34 ± 28.66	55	25.34 ± 33.63	.400
		Week 12	58	19.46 ± 32.43	55	22.49 ± 39.16	.656
Cutometer	Firmness R0 (Uf)	Week 6	57	0.00 ± 0.03	55	0.02 ± 0.05	.014[a]
		Week 12	58	-0.01 ± 04	54[b]	0.02 ± 06	.001[a]
	Elasticity R5 (Ur/Ue)	Week 6	57	0.09 ± 0.10	55	0.08 ± 0.13	.695
		Week 12	58	0.19 ± 0.12	56[b]	0.11 ± 0.18	.008[a]
SIAScope	Collagen	Week 6	57	-6.82 ± 16.24	55	-12.07 ± 17.62	.104
		Week 12	58	20.31 ± 19.40	55	-31.20 ± 33.46	<.001[a]
	Hemoglobin	Week 6	57	1.26 ± 16.49	55	-4.09 ± 17.48	.098
		Week 12	58	-7.09 ± 47.68	55	-3.85 ± 55.07	.739
	Melanin	Week 6	57	-7.19 ± 17.05	55	-11.60 ± 18.98	.199
		Week 12	58	-10.12 ± 38.22	55	-19.83 ± 39.30	.186

[a]The intervention outperformed the placebo, $P \leq .05$.
[b]One participant's (#17) data was not captured at the Week 12 visit due to a technical issue for the Cutometer R0 and R5 (54 participants' data analyzed).

Abbreviations: SD, standard deviation; TEWL, transepidermal water loss

Melanin. The SIAScope measurements revealed a statistically significant improvement in melanin levels for both groups. A modest yet statistically significant improvement in melanin levels ($P = .048$) was found in the intervention group, accompanied by an insignificant change in firmness ($P = .066$) compared to baseline. By contrast, a statistically significant increase in melanin ($P < .001$) had occurred compared to baseline in the placebo group, while firmness ($P = .009$) had a significant worsening.

Skin collagen, firmness, and elasticity. When comparing objective changes between groups based on VapoMeter, MoistureMeterSC, Cutometer MPA 580, and SIAScope assessments, only three statistically significant intergroup differences were found, each of which occurred during the Week 12 visit, as shown in Table 10. The intervention group experienced a statistically significant improvement in skin collagen, firmness, and elasticity compared to the placebo group.

Crow's feet lines and wrinkles. No significant change was seen in the placebo group for average length of crow's feet lines and wrinkles ($P < .094$) and a modest statistically significant reduction occurred for that group in average width ($P < .021$). The intervention group experienced a significant reduction in the average length of crow's feet lines and wrinkles ($P < .010$) as well as an associated improvement in the mean for 90% of participants, together with 100% of participants experiencing a statistically significant improvement in average width of crow's feet lines and wrinkles ($P < .001$), as seen in Table 11.

An improvement in wrinkle severity and fine lines ($P < .001$) occurred in both the intervention group and placebo. A statistically significant decrease occurred in the total number of wrinkles—$P < .002$ for the intervention group and $P < .001$ for the placebo—and in deep lines—$P = .033$ for the intervention group and $P < .001$ for placebo group. Neither group showed a significant improvement in the assessment of changes in emerging lines.

When the intervention group was compared to the placebo by high-resolution image analysis for all 7 instrumental measurements, the only statistically significant improvement seen was in the average width of crow's feet lines and wrinkles , $P < .046$ (Table 12).

Questionnaire. Participants' perceptions of a supplement's value was a secondary measure of this study to gauge its perceived intended benefit. To gauge that value, a questionnaire was administered to participants at Weeks 6 and 12. The questionnaire included 7 questions about the supplement that they had been taking. Responses were scored from 1 to 5; the lowest number indicated complete agreement with a statement, while the highest number indicated complete disagreement. As can be seen in Table 13a, participants in the intervention group rated 4 of 7 statements about the intervention's benefits favorably (≥50%) at Week 6. However, the placebo group rated 5 statements favorably. At the end of the study, each group rated the supplement they were taking favorably for 6 out of 7 statements (Table 13b).

Adverse Events

Daily oral supplementation with the intervention for 12 weeks was well tolerated by all participants. No allergic

Table 11. Intergroup High-resolution Image Analysis of Facial Line Morphologies of Crow's Feet and Wrinkles

Assessment		Time Point	Intervention Group n	Intervention Group Mean ± SD	Intervention Group Mean % Improvement From Baseline Mean	Intervention Group % of Participants with Improvement	Intervention Group *P* Value	Placebo Group n	Placebo Group Mean ± SD	Placebo Group Mean % Improvement From Baseline Mean	Placebo Group % of Participants with Improvement	Placebo Group *P* Value
Crow's Feet Lines /Wrinkles	Average Length (Pixels)	Baseline	20	118.58 ± 22.19				17	122.87 ± 41.70			
		Week 6	17[a]	112.23 ± 12.71	1.13	64.7	.388	13[b]	121.11 ± 31.02	0.72	53.8	.553
		Week 12	20	105.43 ± 20.70	9.97	90.0	.010[b]	17	115.80 ± 38.68	4.52	76.5	.094
	Average Width (Pixels)	Baseline	20	23.84 ± 1.43				17	23.65 ± 1.26			
		Week 6	17[a]	24.04 ± 1.38	NI	52.9	.689	13^	23.72 ± 1.32	NI	38.5	.923
		Week 12	20	22.40 ± 1.60	6.04	100	<.001[b]	17	22.94 ± 1.35	2.95	76.5	.021[b]
	Average Wrinkles Severity	Baseline	20	4448.21 ± 571.12				17	4466.18 ± 634.13			
		Week 6	17[a]	4434.19 ± 466.70	1.13	52.9	.332	13[b]	4493.38 ± 569.96	1.30	38.5	.258
		Week 12	20	4150.22 ± 581.96	6.53	90.0	.001[b]	17	4125.21 ± 703.49	7.66	82.4	.001[b]
	Total Wrinkles Count	Baseline	20	56.30 ± 9.44				17	57.65 ± 7.24			
		Week 6	17[a]	55.12 ± 10.20	4.42	76.5	.065	13[b]	56.62 ± 6.50	3.38	69.2	.106
		Week 12	20	51.00 ± 10.02	9.03	85.0	.002[b]	17	52.41 ± 6.81	9.02	94.1	<.001[b]
	Fine Lines Count	Baseline	20	21.70 ± 10.58				17	22.47 ± 7.28			
		Week 6	17[a]	21.35 ± 9.64	3.77	64.7	.083	13[b]	23.38 ± 7.08	5.09	61.5	.377
		Week 12	20	18.00 ± 8.68	14.67	75.0	.001[b]	17	18.59 ± 6.45	16.27	76.5	<.001[b]
	Deep Lines Count	Baseline	20	3.60 ± 2.78				17	4.53 ± 4.05			
		Week 6	17[a]	3.24 ± 1.99	3.24	41.2	.490	13[b]	3.54 ± 3.28	17.50	53.8	.019[b]
		Week 12	20	2.55 ± 2.28	37.14	60.0	.033[b]	17	2.82 ± 3.30	32.40	70.6	.001[b]
	Emerging Lines Count	Baseline	20	31.50 ± 9.73				17	31.76 ± 9.98			
		Week 6	17[a]	29.82 ± 7.32	NI	52.9	.612	13[b]	29.54 ± 8.70	NI	61.5	.593
		Week 12	20	29.10 ± 8.85	4.19	60.0	.185	17	28.47 ± 7.57	6.86	64.7	.055

[a]Three participants from the intervention group and 4 from the placebo group did not have images and subsequent data at the Week 6 visit due to technical issues (17 participants' data analyzed for the intervention group and 13 participants analyzed for the placebo group at Week 6)
[b]Indicates a statistically significant improvement compared to baseline, $P \leq .05$.

Abbreviations: SD, standard deviation; NI, no improvement.

Table 12. Intergroup Comparison of High-Resolution Image Analyses of Changes in Crow's Feet Lines and Wrinkles

Assessment		Time Point	Intervention Group n	Intervention Group Mean Difference ± SD From Baseline	Placebo Group n	Placebo Group Mean Difference ± SD From Baseline	*P* Value
Crow's Feet Lines/Wrinkles	Average Length (Pixels)	Week 6	17[a]	-4.15 ± 19.26	13[a]	-3.82 ± 22.56	.967
		Week 12	20	-13.15 ± 20.54	17	-7.07 ± 16.35	.323
	Average Width (Pixels)	Week 6	17[a]	0.12 ± 1.24	13[a]	0.02 ± 0.67	.771
		Week 12	20	-1.44 ± 0.92	17	-0.72 ± 1.16	.046[b]
	Average Wrinkles Severity	Week 6	17[a]	-72.29 ± 297.72	13[a]	-67.40 ± 204.51	.958
		Week 12	20	-297.99 ± 352.21	17	-340.96 ± 350.64	.713
	Total Wrinkles Count	Week 6	17[a]	-2.53 ± 5.27	13[a]	-2.23 ± 4.60	.870
		Week 12	20	-5.30 ± 6.51	17	-5.24 ± 2.97	.969
	Fine Lines Count	Week 6	17[a]	-2.24 ± 4.98	13[a]	-1.23 ± 4.83	.582
		Week 12	20	-3.70 ± 4.27	17	-3.88 ± 3.55	.888
	Deep Lines Count	Week 6	17[a]	-0.35 ± 2.06	13[a]	-1.23 ± 1.64	.205
		Week 12	20	-1.05 ± 2.04	17	-1.71 ± 1.83	.310
	Emerging Lines Count	Week 6	17[a]	-1.24 ± 9.86	13[a]	-1.38 ± 9.09	.966
		Week 12	20	-2.40 ± 7.81	17	-3.29 ± 6.57	.708

[a]Three participants in the intervention group and 4 in the placebo group did not have images and subsequent data at the Week 6 visit due to technical issues (17 participants analyzed for the intervention group and 13 analyzed for the placebo group at Week 6)
[b]The intervention outperformed the placebo, $P \leq .05$

Table 13a. Responses to Subjective Questionnaire at Week 6

		Intervention Group							Placebo Group							
		Agree Completely (1) ← →Disagree Completely (5)							Agree Completely (1) ← →Disagree Completely (5)							
Question	n	Response n (%)					% Responding Favorably	n	Response n (%)					% Responding Favorably	Mann Whitney	
1. Reduced the appearance of lines/ wrinkles globally (on the entire face).	58	5 (8.6)	16 (27.6)	28 (48.3)	6 (10.3)	3 (5.2)	36.2	55	3 (5.5)	26 (47.3)	16 (29.1)	6 (10.9)	4 (7.3)	52.8	-0.9236	.355
2. Reduced appearance of lines/ wrinkles in the crow's feet area.	58	4 (6.9)	20 (34.5)	27 (46.6)	4 (6.9)	3 (5.2)	41.4	55	2 (3.6)	24 (43.6)	19 (34.5)	6 (10.9)	4 (7.3)	47.2	-0.0092	.992
3. Improved appearance of skin tone.	58	11 (19.0)	24 (41.4)	16 (27.6)	3 (5.2)	4 (6.9)	60.4	55	5 (9.1)	25 (45.5)	17 (30.9)	6 (10.9)	2 (3.6)	54.6	-1.0077	.313
1. Improved appearance of skin texture/ smoothness.	58	13 (22.4)	25 (43.1)	13 (22.4)	3 (5.2)	4 (6.9)	65.5	55	11 (20.0)	31 (56.4)	8 (14.5)	3 (5.5)	2 (3.6)	76.4	-0.6651	.506
5. Improved facial skin hydration.	58	12 (20.7)	23 (39.7)	17 (29.3)	3 (5.2)	3 (5.2)	60.4	55	14 (25.5)	20 (36.4)	13 (23.6)	5 (9.1)	3 (5.5)	61.9	-0.2314	.817
6. Improved facial skin dryness.	58	10 (17.2)	25 (43.1)	17 (29.3)	3 (5.2)	3 (5.2)	60.3	55	11 (20.0)	24 (43.6)	11 (20.0)	3 (5.5)	6 (10.9)	63.6	-0.1335	.893
7. Improved irritation and redness caused by dryness.	57[a]	7 (12.3)	13 (22.8)	24 (42.1)	4 (7.0)	9 (15.8)	35.1	55	8 (14.5)	18 (32.7)	21 (38.2)	5 (9.1)	3 (5.5)	47.2%	-1.4461	.148

[a]One subject (#83) did not respond to question #7 for the intervention treatment (57 participants' data analyzed)

Table 13b. Subjective Questionnaire Responses at Week 12

		Intervention Group							Placebo Group							
		Agree Completely (1) ← →Disagree Completely (5)							Agree Completely (1) ← →Disagree Completely (5)							
Question	n	Response n (%)					% Responding Favorably	n	Response n (%)					% Responding Favorably	Mann-Whitney	
1. Reduced appearance of lines/ wrinkles globally (on the entire face).	58	5 (8.6)	28 (48.3)	16 (27.6)	6 (10.3)	3 (5.2)	56.9	55	4 (7.3)	27 (49.1)	12 (21.8)	8 (14.5)	4 (7.3)	56.4	-0.3825	0.7021
2. Reduced appearance of lines/ wrinkles in the crow's feet area.	58	4 (6.9)	25 (43.1)	20 (34.5)	5 (8.6)	4 (6.9)	50.0	55	5 (9.1)	28 (50.9)	9 (16.4)	7 (12.7)	6 (10.9)	60.0	-0.4630	0.643
3. Improved appearance of skin tone.	58	6 (10.3)	32 (55.2)	13 (22.4)	4 (6.9)	3 (5.2)	65.5	55	13 (23.6)	20 (36.4)	15 (27.3)	3 (5.5)	4 (7.3)	60.0	-0.3884	0.697
4. Improved appearance of skin texture/ smoothness	58	13 (22.4)	29 (50.0)	7 (12.1)	7 (12.1)	2 (3.4)	72.4	55	13 (23.6)	24 (43.6)	10 (18.2)	4 (7.3)	4 (7.3)	67.3	-0.2602	0.794
5.Improved facial skin hydration.	58	16 (27.6)	26 (44.8)	5 (8.6)	9 (15.5)	2 (3.4)	72.4	55	13 (23.6)	22 (40.0)	11 (20.0)	5 (9.1)	4 (7.3)	63.6	-0.7381	0.460
6. Improved facial skin dryness.	58	15 (25.9)	25 (43.1)	5 (8.6)	9 (15.5)	4 (6.9)	69.0	55	14 (25.5)	21 (38.2)	9 (16.4)	5 (9.1)	6 (10.9)	63.6	-0.3219	0.747
7. Improved irritation and redness caused by dryness.	58	9 (15.5)	21 (36.2)	17 (29.3)	4 (6.9)	7 (12.1)	51.7	55	8 (14.5)	16 (29.1)	23 (41.8)	6 (10.9)	2 (3.6)	43.6	-0.2491	0.803

responses or adverse events were reported by any of the participants.

DISCUSSION

This study found that 12 weeks of daily supplementation with the intervention reduced visible and measurable, age-dependent signs on the face, including crow's feet lines and depth of wrinkles.

The current study addressed the need for a controlled clinical trial of a proprietary supplement, given the promising results reported by Schwartz and Park[10] in their pilot, open-label study. The statistically significant increase in dermal collagen following 12 weeks of supplementation with the intervention ($P < .001$) in the current study supports their findings on the supplement's efficacy as reported.[10] In the current study, 86.2% of women in the intervention group

showed an improvement in collagen levels at 12 weeks compared to baseline, while only 7.3% showed an improvement in the placebo group.

Schwartz and colleagues also reported that the same one gram of the intervention taken daily over 12 weeks led to a significant reduction in skin dryness and scaling and global lines and wrinkles based on visual and tactile inspection. In the current study, statistically similar results were found at the same dose at Week 12 based on expert visual grading, including the following outcomes: an improvement in the average length, average width, severity, and number of crow's feet lines and wrinkles, along with a reduction in fine line depth and number of fine lines. However, when performing intergroup analysis of the data, the placebo group experienced many of the same statistically significant benefits except for an improvement in the average length of crow's feet lines and wrinkles seen in the intervention group ($P < .010$) that did not occur in the placebo group ($P = .094$).

As the dermal collagen network is responsible for controlling water status in the skin, two measures of skin related hydration were assessed in the current study to measure transepidermal water loss as well as skin surface hydration. The study found a statistically significant mean percentage reduction in skin dryness ($P < .001$) and erythema ($P < .002$) after 12 weeks compared to baseline values in the intervention group. For the placebo group the degree of erythema did not improve ($P = .255$), while skin dryness did ($P < .001$). Between-group analysis showed no significant difference in skin dryness between the two groups because both improved to the same degree.

A limited number of studies suggest that maintaining optimal levels of collagen through supplementation with collagen peptides can delay[56] the appearance and severity of facial wrinkles associated with aging, believed due to inhibiting platelet release.[57] As mentioned earlier, the intervention contains ingredients derived from chicken sternal cartilage that include a naturally occurring matrix of collagen type-II peptides, hyaluronic acid (HA), and chondroitin sulfate (CS).

Skin is organized into 3 anatomical regions: the outermost epidermis, where desquamation of squamous cells occurs daily in response to physical insult and UV photo-damage; the innermost insulating hypodermal layer, which is rich in fat cells; and a thicker elastic layer that varies in thickness between 1-3 mm in diameter, characterized by a network of structural tissues that contain blood and lymph vessels, mast cells, fibroblasts as well as, collagen, elastin, GAG, HA, and CS. The importance of HA relates to the contribution of this high-molecular-weight biopolysaccharide of the mesh-like structural framework of the dermis together with its ability to promote fibroblast proliferation.[37,58] Thereby, it plays an important role structurally in maintaining the skin's extracellular matrix at desirable moisture levels owing to HA's unique capacity to retain water.

Hence, given its hygroscopic properties, it seemed reasonable for the current research team to expect that the intervention, since it contains HA, would lead to a reduction in skin dryness and an improvement in skin moisture, even in participants with sufficient quantities of senescent skin cells in the dermis resulting from natural processes and photoaging. This biopolymer also is able to interact with a number of receptors to activate signaling cascades that influence cell migration, gene expression, and proliferation.[31,59,60] However, assessments that the current research team made for moisture content and dryness of the skin by the Vapometer and MoistureMeter found no such statistically significant improvement in the intervention group or following the analysis between groups. This finding suggests that a longer period of time may be needed than 12 weeks to demonstrate an unequivocal benefit for the intervention to improve moisture content and skin dryness. Fraser and colleagues[33] reported that HA has a half-life of 3 to 5 minutes in the blood and less than a day in the skin. Possibly a more frequent dosing schedule for the intervention, given HA's half-life, would allow levels to increase in healthy skin cells. The lack of statistical changes observed in the current study for skin hydration mirrors the findings reported in a recent study of porcine-derived supplements.[14]

A study limitation is the lack of a biochemical analysis of plasma hydroxyproline content, a major component of collagen, which limits the ability to conclude that an increase in collagen synthesis occurred.

The viscoelastic property of the skin is another factor that contributes to the health of the dermal collagen network. The nature of this network in the dermis layer dictates how skin responds when deformed physically or challenged by environmental stress. Healthy dermal viscoelasticity is the result of a lack of proper collagen fibril content and interconnectivity and inclusivity of GAG, HA and CS, in this network.

Aging of the skin is a complex process in which senescent cells play a central role. The nucleus of senescent cells is characterized by senescence-associated heterochromatin foci (SAHF) and DNA segments with chromatin that accelerate aging. Its symptoms include visible rhytides characterized by creases, deepening crevices or furrows, of the skin. Senescent induction of skin cells can be promoted by multiple factors including loss of body mass, advanced glycation end products, excessive exposure to ultraviolet radiation, addiction to tobacco products, poor hydration, habitual facial expressions, and other factors.[61]

Mitochondria is hypothesized to also play a key role in the aging process due to the generation of excess endogenous ROS production during respiration that leads to cellular apoptosis.[62] Among four mitochondrial complexes (I- IV), mitochondrial complex II has been shown to produce ROS and play a role in generating ROS in human skin cells.[63] The rate of complex II enzymatic activity per unit of mitochondria cultured cells has been shown to decrease in an age-dependent manner in adult human skin fibroblasts. This decrease significantly inhibits transcript expression by fibroblasts of the succinate dehydrogenase complex, resulting

in a decrease in complex II activity.[64] This decrease was only found to occur in senescent skin fibroblasts. As these senescent skin cells increase in number, they would have a disruptive effect on skin tissue and its appearance as the level of ROS and RNS production increases.[61]

As one of the most complex regions of the body, the face experiences interactions that occur between ligaments, muscles, fat, and bone that can affect the appearance of facial skin.[65] The structural integrity of the skin relies on maintaining optimal hydration, vasculature and structural support during aging. Of the dry weight of skin, 70% to 80% is primarily composed of type-I collagen fibrils critical to supporting the extracellular matrix of the skin.[66] This matrix is synthesized by dermal fibroblasts producing glycosaminoglycans, collagen, and elastin fibers. Fragmentation of skin collagen, in particular, has been shown to increase over the years until it affects the function of dermal fibroblasts resulting in changes in the skin's structural integrity and appearance. Collapsed fibroblasts result in lowered levels of collagen and higher levels of collagen-degrading enzymes, as the skin ages, leading to fragmentation of collagen culminating in the loss of structural integrity and impairment of fibroblast function.[67] With age, replenishment of collagen, along with elastin and glycosaminoglycans (GAG) declines. The resulting connective tissue manifests as wrinkled skin and/or dry, flaky skin. Wrinkles occur when the sulcus cutis (grooves) already present in the skin become deeper.

As the intervention contains all 3 dermal collagen skin constituents, the research team asked whether daily ingestion over 12 weeks would influence improvements in the viscoelasticity behavior of the skin, as assessed by use of the Cutometer device. The results found a statistically significant improvement in skin elasticity ($P < .001$) in the intervention group from baseline compared to that of the placebo group. The magnitude of increase in skin elasticity after 12 weeks was also significant compared to placebo. This finding agrees with previous studies in which supplementation with hydrolyzed collagen increased collagen levels in rats as well as collagen content in clinical studies in older females.[14] In a study by Zague,[13] an improvement in skin elasticity was reported following supplementation with hydrolyzed collagen, similar to that observed years later in the Proksch[14] study.

Although the intervention had a measurable impact on improving the function of the dermal collagen network in the current study, when improvement in viscoelasticity of the skin in the placebo group was evaluated, the intergroup analysis failed to show a statistically significant difference between groups. Nor did a statistically significant difference in improvement between groups occur in the indirect measurement of collagen in the skin or in skin hemoglobin and melanin content, as assessed objectively by use of the SIAscope. These results, although disappointing, may suggest that in future studies the dose of one gram a day may be insufficient to yield statistically significant improvements over placebo. Further, the manufacturers recommended dosage regime might want to take into consideration the age of the consumer because skin-elasticity studies of women have shown that an inverse correlation existed with age, suggesting higher levels may be needed for optimal performance.[68]

Participants undergoing facial high-resolution image analysis of crow's feet facial morphologies showed a statistically significant average improvement in wrinkle width of 6.04% following the intervention ($P < .001$) for 12 weeks, compared to a less significant ($P \leq .021$) change in the placebo group. This implies that chronic supplementation with the intervention for extended periods of time may improve dermal elasticity to help reduce physical signs of photoaging. Overall, a lag in the associated equilibration phase appears to occur for any beneficial effects to be observed with the intervention on skin conditions, suggesting the need for longer-term controlled trials of at least six months duration.

The participants completed a questionnaire to determine their perceptions of the value of supplementation with the supplement they were given, but it revealed no significant differences in responses to the 7 statements either at the 6- or 12-week visits. Participants in the intervention group responded favorably (≥50%) to six out of seven statements, ranging from 50% to 72.4%, at the end of the study. Similar perceptions of value were seen in the placebo group for 6 of the statements, ranging in favorable responses that ranged from 56.4% to 67.3%, with the exception of the statement on whether the supplement improved irritation and redness caused by dryness, for which only 43.6% reported a favorable response. A Mann-Whitney analysis of the questionnaire's results revealed no statistically significant difference in responses from participants using either the intervention or placebo.

A number of weaknesses exist in the study. The outcomes of this study cannot be applied to adult males, because only adult females participated in the study, nor could they apply to adults or children under the age of 39. As the current study was the first controlled clinical trial of the intervention, a longer study of at least 6 months might show different results. Whether the time period in which the study was performed affected the outcomes is unknown, because the study was carried out from mid-May to mid-September when participants would have been exposed to higher levels of UV radiation than had the study been conducted when lower levels occur.

Whether higher levels of UV exposure could have impacted the results of the study could not be determined, other than that none of the participants showed evidence of excessive sun exposure based on visual grading or review of their diaries during the 12-week study. The lack of biochemical analysis of participant's plasma level of hydroxyproline at baseline and the end of the study, given that this protein is a major component of collagen, limits the current study's ability to conclude that collagen synthesis increased in response to the intervention compared to placebo, since the SIAScope provides only an indirect measure of the skin-collagen content.

CONCLUSIONS

The intent of this study was to assess the efficacy of repeated daily oral administration of Biocell Collagen (BCC) as a dietary supplement to improve markers of facial skin aging. The secondary objective was to assess the tolerance of the intervention when taken according to the manufacturer's instructions.

Under the conditions of this study, use of both the intervention and the placebo led to significant improvements in the appearance and condition of the facial skin, as evaluated by expert clinical grading, instrumental measures, and image analysis. Additionally, participants' perceptions revealed a significantly positive perception of supplement effectiveness on the facial skin after 6 and 12 weeks of use.

Comparative between-group analysis of results for the intervention and placebo revealed that they performed at parity for the majority of subjective and objective evaluations. However, after 12 weeks of repeated use, the intervention outperformed the placebo by reducing facial lines and wrinkles, as evaluated by clinical grading and image analysis for crow's feet width as well as firmness and elasticity. Skin moisture did not improve in either group, while mean hydration improved in both groups, likely due to daily increased water intake in order to swallow the supplement or placebo.

The intervention was well tolerated by participants, as indicated by expert visual grading of the facial skin, with significant improvements noted at the end of the study in mean dryness and erythema for participants using the intervention and in mean dryness for participants given a placebo. This tolerance to the intervention and the lack of any reported adverse events provides further evidence for the intervention's safety as a dietary supplement.

AUTHOR DISCLOSURE STATEMENT

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